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(54) Title: ONCOGENIC OSTEOMALACIA-RELATED GENE 1

(57) Abstract: This invention provides isolated polynucleotides encoding an oncogenic osteomalacia-related factor and polypeptides encoded by this polynucleotide. Expression systems, including gene delivery vehicles such as liposomes and vectors, and host cells containing the polynucleotides are further provided by this invention. The present invention also provides proteins encoded by the polynucleotides and antibodies that specifically recognize and bind to these proteins. In one embodiment the proteins that modulate bone mineralization and phosphate metabolism as characterized by the methods described herein. The present invention also provides methods for modulating bone mineralization activity and phosphate metabolism and for treating diseases related to abnormal bone mineralization and abnormal phosphate metabolism.

Of several known bone diseases associated with hypophosphataemia, X-linked hypophosphataemia rickets (HYP) and oncogenic osteomalacia (OOM) have been extensively studied in recent years. Rowe (1994) *Hum. Genet.* 94:457-467. HYP and OOM, although one inherited and the other acquired, have very similar clinical features.

5 Both are characterized by symptoms such as hypophosphataemia, phosphaturia, and low serum concentrations of 1,25-dihydroxyvitamin D. Inadequate phosphate level leads to defective skeletal mineralization, which in turn causes deformed bones (rickets) or bone softening (osteomalacia).

10 Biological and clinical studies of hypophosphataemia- or hyperphosphataemia-related syndromes have been focused on understanding the molecular mechanism of phosphate uptake. Recent developments have provided several lines of evidence suggesting the existence of a humoral factor or factors specifically involved in renal phosphate transport regulation. Kumar (1997), *supra*. In HYP studies, a novel gene, *PEX*, has been associated with the hypophosphataemia phenotype. The *PEX* gene

15 shares strong homology to a family of membrane-bound endopeptidases. Thus it is postulated that the *PEX* protein functions in cellular processing of putative hormone substrates. Rowe (1997) *Exp. Nephrol.* 5:355-363. OOM can be caused by a variety of histologically distinct tumors, mainly of mesenchymal origin (haemangiopericytomas). Removal of the tumor from the OOM patients promptly reverses defective symptoms

20 and results in complete cure of the bone disease, indicating a tumor-secreted circulating factor capable of inhibiting renal phosphate transport.

25 Despite the well-documented circumstantial evidences for one or more humoral factors specifically involved in phosphate homeostasis regulation, the putative factor, "phosphatonin," is yet to be identified. Several attempts to purify the factor or clone the gene encoding a protein having phosphatonin activity have been unsuccessful, in part due to difficulties in maintaining secretion levels of the putative factor in established tumor cultures.

30 In addition to phosphate regulation, it is likely that some effects of OOM as well as certain defects in bone mineralization are mediated by factors that directly interfere or promote bone metabolism. These could include developmental regulators of bone formation as well as factors that mediate bone mineral homeostasis. Such factors provide targets for improved therapies for a wide range of conditions including,

osteoporosis, osteomalacia, rickets, hypophosphatasia, Falconin syndrome and renal osteodystrophy.

The highly interactive pathways that govern phosphate metabolism and bone mineralization may also be influenced indirectly by polypeptide factors functioning to 5 control protein synthesis, processing and secretion. For example, OOM induced changes may alter phosphate metabolism and bone mineralization by altering the balance of factors containing heparin sulfate or other glycosaminoglycans by increasing or decreasing the levels of lysosomal proteases. Identification of such factors will provide useful insights into phosphate regulation disorders.

10 Thus, there exists a need to identify genes differentially expressed in neoplastic cells associated with OOM, particularly those responsible for regulating renal phosphate transport, bone mineralization and protein metabolism. The analysis of gene expression pattern specific to the cells of interest not only leads to the identification of genes corresponding to homeostasis-regulating activity, but also provides molecular 15 information about gene activities related to other tumor-associated disease states. For example, tumor cells associated with OOM might be considered as a source of novel angiogenic factors or could be used to compare gene expression with different types of tumors. The identification of tumor-derived regulating factors can also help diagnosing and treating non-cancerous diseases with irregular phosphate homeostasis, such as renal 20 failures and inherited rickets. Furthermore, the identification of factors that directly control bone formation and metabolism will provide important tools for therapeutic intervention in bone disease. Ultimately this will lead to the understanding of the mechanisms involved in phosphate metabolism and osteogenesis.

25

#### DESCRIPTION OF THE INVENTION

This invention provides isolated polynucleotides encoding oncogenic osteomalacia-related genes (OOM) and polypeptides encoded by the polynucleotides. Polynucleotides of the invention are intended to include DNA, cDNA, RNA and genomic DNA. Expression systems, including gene delivery vehicles such as liposomes 30 and vectors, and host cells containing the polynucleotides are further provided by this invention.

The present invention also provides proteins encoded by the polynucleotides. In one embodiment, the proteins have oncogenic osteomalacia-related activity, which can be detected by using the methods described herein.

Additionally, nucleic acid probes and primers that hybridize to invention 5 polynucleotides are provided, as well as isolated nucleic acids comprising unique, expressed gene sequences.

The present invention further includes antisense oligonucleotides, antibodies, hybridoma cell lines and compositions containing same.

The present invention also provides methods of monitoring gene expression 10 using invention polynucleotides.

The methods of monitoring gene expression are useful for detecting a cell expressing oncogenic osteomalacia-related polypeptide and for detecting a neoplastic cell associated with oncogenic osteomalacia.

This invention further provides methods for modulating the expression of the 15 inventive polynucleotides, for altering the activity of the proteins encoded by the polynucleotides, and for treating symptoms of phosphate transport related diseases and diseases characterized by abnormal bone mineralization. These diseases include but are not limited to, oncogenic osteomalacia, X-linked hypophosphataemia rickets, rhabdomyolysis, osteoporosis, cardiamyopathy, tumoral calcinosis and renal failure.

This invention also provides a method for screening for candidate agents that 20 modulate the expression of a polynucleotide of the invention or its complement, by contacting a test agent with a neoplastic cell associated with oncogenic osteomalacia and monitoring expression of the polynucleotide, wherein the test agent which modifies the expression of the polynucleotide is a candidate agent.

25

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 (SEQ ID NO:1) is a polynucleotide sequence encoding an oncogenic 30 osteomalacia-related protein (OOM-1). The polynucleotide contains 1946 nucleotides and has a reading frame that stretches from position 52 through 1629. There is an open reading frame from nucleotide 52 through 1626. The initiating ATG and termination codon is underlined.

Figure 2 is the peptide sequence of an oncogenic osteomalacia-related protein (OOM-1). A predictive signal peptide sequence encoding 16 amino acids is underlined.

Cleavage occurs between amino acids 16 and 17. The amino acid sequence is SEQ ID NO:2.

#### MODE(S) FOR CARRYING OUT THE INVENTION

5 Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

10

#### Definitions

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, organic chemistry, microbiology, cell biology and recombinant DNA. These methods are described in the 15 following publications. See, e.g., Sambrook, et al. MOLECULAR CLONING: A LABORATORY MANUAL, 2<sup>nd</sup> edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds, (1987)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.); "PCR: A PRACTICAL APPROACH" (M. MacPherson, et al., IRL Press at Oxford University Press (1991)); PCR 2: A PRACTICAL APPROACH (M.J. 20 MacPherson, B.D. Hames and G.R. Taylor eds. (1995)); ANTIBODIES, A LABORATORY MANUAL (Harlow and Lane, Eds. (1988)); and ANIMAL CELL CULTURE (R.I. Freshney, ed. (1987)).

25 As used in the specification and claims, the singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "comprising" is intended to mean that the compositions and methods 30 include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering

the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

The terms "polynucleotide" and "nucleic acid molecule" are used interchangeably to refer to polymeric forms of nucleotides of any length. The 5 polynucleotides may contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Nucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term "polynucleotide" includes, for example, single-, double-stranded and triple helical molecules, a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched 10 polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid molecule may also comprise modified nucleic acid molecules.

A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed 15 and translated.

A "gene product" refers to the amino acid (*e.g.*, peptide or polypeptide) generated when a gene is transcribed and translated.

As used herein a second polynucleotide "corresponds to" another (a first) polynucleotide if it is related to the first polynucleotide by any of the following 20 relationships:

1) The second polynucleotide comprises the first polynucleotide and the second polynucleotide encodes a gene product.

2) The second polynucleotide is 5' or 3' to the first polynucleotide in cDNA, RNA, genomic DNA, or fragment of any of these polynucleotides. For example, a 25 second polynucleotide may be a fragment of a gene that includes the first and second polynucleotides. The first and second polynucleotides are related in that they are components of the gene coding for a gene product, such as a protein or antibody. However, it is not necessary that the second polynucleotide comprises or overlaps with the first polynucleotide to be encompassed within the definition of "corresponding to" 30 as used herein. For example, the first polynucleotide may be a fragment of an untranslated region of the second polynucleotide, or it may comprise a promoter sequence for the gene comprising the tag. The first and second polynucleotide may be fragment of a gene coding for a gene product. The second polynucleotide may be an

exon of the gene while the first polynucleotide may be an intron of the gene.

3) The second polynucleotide is the complement of the first polynucleotide.

A "foreign polynucleotide" is a DNA sequence that is foreign to the cell, vector or a position therein, wherein it is placed.

5 A "sequence tag" or "tag" or "SAGE tag" is a short oligonucleotide containing defined nucleotide sequence that occurs in a certain position of a gene transcript. The length of a tag is generally under about 20 nucleotides, preferably between 9 to 15 nucleotides, and more preferably 10 nucleotides. The tag can be used to identify the corresponding transcript and gene from which it was transcribed. A tag can further 10 comprise exogenous nucleotide sequences to facilitate the identification and utility of the tag. Such auxiliary sequences include, but are not limited to, restriction endonuclease cleavage sites and well-known primer sequences for sequencing and cloning.

15 A sequence is the complement or is complementary to another sequence if they are related by the base-pairing rules. For example, in DNA, a sequence A-G-T in one strand is complementary to T-C-A in the other strand. A given sequence defines the complementary sequence.

As used herein, the term "modulate" means to alter or modify a process or biological function associated with angiogenesis.

20 The term "peptide" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, *e.g.* ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical 25 isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

The term "cDNAs" refers to complementary DNA, that is mRNA molecules present in a cell or organism made into cDNA with an enzyme such as reverse 30 transcriptase. A "cDNA library" is a collection of all of the mRNA molecules present in a cell or organism, all turned into cDNA molecules with the enzyme reverse transcriptase, then inserted into "vectors."

A "probe" when used in the context of polynucleotide manipulation refers to an

oligonucleotide that is provided as a reagent to detect a target potentially present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, 5 fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes.

A "primer" is a short polynucleotide, generally with a free 3' -OH group that binds to a target or "template" potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A "polymerase chain reaction" ("PCR") is a reaction in 10 which replicate copies are made of a target polynucleotide using a "pair of primers" or a "set of primers" consisting of an "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and taught, for example in "PCR: A PRACTICAL APPROACH" (M. MacPherson *et al.*, IRL Press at 15 Oxford University Press (1991)). All processes of producing replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "replication." A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses. Sambrook *et al.*, *supra*.

A "promoter" is a region on a DNA molecule to which an RNA polymerase 20 binds and initiates transcription. In an operon, the promoter is usually located at the operator end, adjacent but external to the operator. The nucleotide sequence of the promoter determines both the nature of the enzyme that attaches to it and the rate of RNA synthesis.

An "activated cell" is one which naturally or has been modified to express an 25 amount of serine protease 11 effective to activate the gene and the expression product of this invention.

The term "genetically modified" means containing and/or expressing a foreign gene or nucleic acid sequence which in turn, modifies the genotype or phenotype of the cell or its progeny. "Foreign nucleic acid" includes, but is not limited to promoters, 30 enhancers and gene activators. For example, a genetically modified cell includes a cell that contains a polynucleotide encoding oncogenic osteomalacia-related polypeptide in its native environment but not expressed and expression has been turned on or the level of expression has been enhanced or lowered by the upstream insertion of a gene

activator.

As used herein, "expression" or "expressed" refers to the process by which polynucleotides are transcribed into mRNA or by which transcription is enhanced. In another embodiment, the RNA is translated into peptides, polypeptides, or proteins. If 5 the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA, if an appropriate eukaryotic host is selected.

"Differentially expressed" as applied to a gene, refers to the differential production of the mRNA transcribed from the gene or the protein product encoded by the gene. A differentially expressed gene may be overexpressed or underexpressed as 10 compared to the expression level of a normal or control cell. In one aspect, it refers to a differential that is 3 times, preferably 5 times, or preferably 10 times higher or lower than the expression level detected in a control sample. The term "differentially expressed" also refers to nucleotide sequences in a cell or tissue which are expressed where silent in a control cell or not expressed where expressed in a control cell.

15 "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi- 20 stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

Hybridization reactions can be performed using traditional hybridization 25 techniques under different stringency. In general, a low stringency hybridization reaction is carried out at about 40°C in 10 X SSC or a solution of equivalent ionic strength/temperature. A moderate stringency hybridization is typically performed at about 50°C in 6 X SSC, and a high stringency hybridization reaction is generally performed at about 60°C in 1 X SSC. Alternatively, TMAC hybridization technology 30 can be used for hybridization reactions probed with pooled oligonucleotides such as the SAGE tags. The advantage of using TMAC hybridization is that the reaction condition is not dependent on the G+C content of the oligonucleotide, and the melting temperature is determined only by the length of the oligomers to be used.

When hybridization occurs in an anti-parallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary." A double-stranded polynucleotide can be "complementary" or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second.

5 "Complementarity" or "homology" (the degree that one polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to form hydrogen bonding with each other, according to generally accepted base-pairing rules. A polynucleotide that is 100% complementary to a second polynucleotide are understood to be "complements" of each other.

10 A "composition" is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label or a pharmaceutically acceptable carrier) or active, such as an adjuvant.

15 A "pharmaceutical composition" is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

20 As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin, REMINGTON'S PHARM. SCI., 15th Ed., Mack Publ. Co., Easton, PA (1975).

25 An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

A "subject," "individual" or "patient" is used interchangeably herein, which refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

30 A "control" is an alternative subject or sample used in an experiment for comparison purpose. A control can be "positive" or "negative." For example, where the purpose of the experiment is to determine a correlation of an altered expression level of a gene with a particular type of cancer, it is generally preferable to use a positive

control (a subject or a sample from a subject, carrying such alteration and exhibiting syndromes characteristic of that disease), and a negative control (a subject or a sample from a subject lacking the altered expression and clinical syndrome of that disease).

5 A “gene delivery vehicle” is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, cationic liposomes, viruses, such as baculovirus, adenovirus, adeno-associated virus, and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for 10 simple protein expression.

A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral vectors include retroviral vectors, adenovirus vectors, adeno-associated virus vectors and the like. In aspects where gene transfer is mediated 15 by a retroviral vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and the inserted polynucleotide. As used herein, “retroviral mediated gene transfer” or “retroviral transduction” carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its 20 genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

25 Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form that integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus.

In aspects where gene transfer is mediated by a DNA viral vector, such as an 30 adenovirus (Ad) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a polynucleotide to be inserted. Adenoviruses (Ads) are a relatively well characterized, homogenous group of viruses, including over 50 serotypes. (see, e.g., WO 95/27071). Ads are easy to grow

and do not require integration into the host cell genome. Recombinant Ad-derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. (see, WO 95/00655; WO 95/11984). Wild-type AAV has high infectivity and specificity integrating into the host cells 5 genome. See, Hermonat and Muzyczka (1984) *Proc. Natl. Acad. Sci. USA* **81**:6466-6470; and Lebkowski et al. (1988) *Mol. Cell. Biol.* **8**:3988-3996.

Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from 10 sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or 15 translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression.

Gene delivery vehicles also include several non-viral vectors, including DNA/liposome complexes, and targeted viral protein DNA complexes. Liposomes that also comprise a targeting antibody or fragment thereof can be used in the methods of 20 this invention. To enhance delivery to a cell, the nucleic acid or proteins of this invention can be conjugated to antibodies or binding fragments thereof which bind cell surface antigens, e.g., TCR, CD3 or CD4.

Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable 25 conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a 30 termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of

human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; stabilizing elements 3' to the inserted polynucleotide, and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Other means are well known and available in the art.

“Host cell” is intended to include any individual cell or cell culture which can be or have been recipients for vectors or the incorporation of exogenous polynucleotides, polypeptides and/or proteins. It also is intended to include progeny of a single cell, and 10 the progeny may not necessarily be completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. The cells may be prokaryotic or eukaryotic, and include but are not limited to bacterial cells, yeast cells, plant cells, insect cells, animal cells, and mammalian cells, e.g., murine, rat, simian or human.

15 An “antibody” is an immunoglobulin molecule capable of binding an antigen. As used herein, the term encompasses not only intact immunoglobulin molecules, but also anti-idiotypic antibodies, mutants, fragments, fusion proteins, humanized proteins and modifications of the immunoglobulin molecule that comprise an antigen recognition site of the required specificity.

20 As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA, if an appropriate eukaryotic host is selected. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription 25 initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the *lac* promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Sambrook, et al. (1989) *supra*). Similarly, an eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon 30 AUG, and a termination codon for detachment of the ribosome. Such vectors can be obtained commercially or assembled by the sequences described in methods well known in the art, for example, the methods described below for constructing vectors in general.

A “subject” is a vertebrate, preferably a mammal, more preferably a human.

Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

A "control" is an alternative subject or sample used in an experiment for comparison purpose. A control can be "positive" or "negative."

5 As used herein, the term "cytokine" refers to any one of the numerous factors that exert a variety of effects on cells, for example, inducing growth or proliferation. Non-limiting examples of cytokines which may be used alone or in combination in the practice of the present invention include, interleukin-2 (IL-2), stem cell factor (SCF), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 12 (IL-12), G-CSF, granulocyte 10 macrophage-colony stimulating factor (GM-CSF), interleukin-1 alpha (IL-1), interleukin-11 (IL-11), MIP-1, leukemia inhibitory factor (LIF), c-kit ligand, thrombopoietin (TPO) and flt3 ligand. The present invention also includes culture conditions in which one or more cytokine is specifically excluded from the medium. Cytokines are commercially available from several vendors such as, for example, 15 Genentech (South San Francisco, CA), Amgen (Thousand Oaks, CA), R&D Systems (Minneapolis, MN) and Immunex (Seattle, WA). It is intended, although not always explicitly stated, that molecules having similar biological activity as wild-type or purified cytokines (e.g., recombinantly produced or muteins thereof) are intended to be used within the spirit and scope of the invention.

20 The term "culturing" refers to the *in vitro* propagation of cells or organisms on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (i.e., morphologically, genetically, or phenotypically) to the parent cell. By "expanded" is meant any proliferation or division of cells.

25 The terms "cancer," "neoplasm," and "tumor," used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells (that is, cells obtained from near the site of malignant transformation) can be readily distinguished from non-cancerous cells by well-established techniques, particularly 30 histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but also any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and *in vitro* cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid

tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass; *e.g.*, by such procedures as CAT scan, magnetic resonance imaging (MRI), X-ray, ultrasound or palpation. Biochemical or immunologic findings alone may be insufficient to meet this definition. Tumor cells often express antigens that are tumor specific. The term "tumor associated antigen" or "TAA" refers to an antigen that is associated with or specific to a tumor.

As used herein, "solid phase support" is not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, 10 glass beads, cotton, plastic beads, alumina gels. A suitable solid phase support may be selected on the basis of desired end use and suitability for various synthetic protocols. For example, for peptide synthesis, solid phase support may refer to resins such as polystyrene (*e.g.*, PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), POLYHIPE® resin (obtained from Aminotech, Canada), polyamide resin (obtained from 15 Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (TentaGel™, Rapp Polymere, Tubingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Bioscience, California). In a preferred embodiment for peptide synthesis, solid phase support refers to polydimethylacrylamide resin.

A "transgenic animal" refers to a genetically engineered animal or offspring of 20 genetically engineered animals. The transgenic animal may contain genetic material from at least one unrelated organism (such as from a bacteria, virus, plant, or other animal) or may contain a mutation which interferes with expression of a gene product.

The term "oncogenic osteomalacia" (OOM), "oncogenic hypophosphataemic osteomalacia" (OHO), or "tumor-associated osteomalacia" refers to a tumor-acquired 25 syndrome characterized mainly by hypophosphataemia, hyperphosphaturia, abnormally low serum level of 1,25-dihydroxyvitamin D, and osteomalacia. Tumors associated with OOM are mainly of mesenchymal origin such as haemangiopericytomas, although carcinoma of prostate and lung, fibrous dysplasia of bone, linear sebaceous naevus syndrome, neurofibromatosis, and oat cell carcinoma are also associated with OOM. 30 Thus, the OOM syndrome can be described as having a paraneoplastic aetiology. Surgical removal of the tumor in a patient often results in a complete or near-complete resolution of biochemical and clinical defects associated with OOM.

The term "phosphatonin" refers to a polypeptide humoral factor specifically involved in the regulation of phosphate homeostasis.

A factor with "phosphatonin activity" down regulates the renal reabsorption of inorganic phosphate. Phosphatonin activity incorporates the combined function of 5 phosphatonin and additional modulating factors.

The term "bone mineralization" as used herein refers broadly to the processes of bone formation and homeostasis. Thus, bone mineralization includes the formation of solid bone structures that occurs during growth, development and wound healing and also to the normal maintenance of differentiated bone structures.

10

### Polynucleotides

The present invention provides isolated polynucleotides of a oncogenic osteomalacia-related gene and analogs and variants thereof. In addition the invention provides for fragments of oncogenic osteomalacia-related gene, gene delivery vehicles 15 comprising this gene, probes and primers derived from this gene and anti-sense polynucleotides based on the polynucleotide sequence of the gene.

The polynucleotides of this invention comprise an isolated polynucleotide encoding oncogenic osteomalacia-related polypeptide (OOM1) or an analog or variant thereof, wherein the polynucleotide comprises or corresponds to a polynucleotide 20 selected from the group consisting of:

- a. a polynucleotide comprising or corresponding to SEQ ID NO:1 or its complement;
- b. a polynucleotide that hybridizes under stringent conditions to the polynucleotide of SEQ ID NO:1 or its complement;
- c. a polynucleotide having greater than 80% sequence homology to SEQ ID 25 NO:1 or its complement;
- d. a polynucleotide having greater than 90% sequence homology to SEQ ID NO:1 or its complement; and
- e. a polynucleotide having greater than 95% sequence homology to SEQ ID 30 NO:1; or its complement.
- f. a polynucleotide comprising nucleotides 52 -1629 of SEQ ID NO:1 or its complement;

- g. a polynucleotide that hybridizes to nucleotides 52-1629 of SEQ ID NO:1 or its complement;
- h. a polynucleotide having greater than 80% sequence homology to nucleotides 52-1629 of SEQ ID NO:1 or its complement;
- 5 i. a polynucleotide having greater than 90% sequence homology to nucleotides 52-1629 of SEQ ID NO:1 or its complement;
- j. a polynucleotide having greater than 95% sequence homology to nucleotides 52-1629 of SEQ ID NO:1 or its complement;
- 10 k. a polynucleotide comprising nucleotides 100 -1629 of SEQ ID NO:1 or its complement;
- l. a polynucleotide that hybridizes to nucleotides 100-1629 of SEQ ID NO:1 or its complement;
- m. a polynucleotide having greater than 80% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement;
- 15 n. a polynucleotide having greater than 90% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement; and
- o. a polynucleotide having greater than 95% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement.

20 In an another aspect of this invention, the isolated polynucleotide encodes an oncogenic osteomalacia-related polypeptide, the polypeptide having the amino acid sequence of SEQ ID NO:2 or an analog thereof having conservative amino acid substitutions. In a further aspect, the isolated polynucleotide of this invention encodes oncogenic osteomalacia-related mutein polypeptide, the mutein polypeptide having the amino acid sequence of SEQ ID NO:2 or an analog thereof having non-conservative 25 amino acid substitutions.

30 Oncogenic osteomalacia-related genes include genes that have been identified to be over-expressed or under-expressed relative to control tumors (histologically similar tumors that are not associated with OOM). Genes that are up-regulated or down-regulated in oncogenic osteomalacia may encode proteins involved in several distinct biochemical pathways. These include phosphate regulation, bone mineralization, and protein synthesis, proccsing and secretion.

The regulation of phosphate metabolism plays a central role in mediating the symptoms of oncogenic osteomalacia. Genes whose expression is altered in OOM

tumors can effect phosphate metabolism through a variety of mechanisms. For example, the tumor may directly produce increased amounts of phosphatonin, a secreted humoral factor whose activity includes inhibition of phosphate re-absorption in the kidney. Alternatively the OOM tumor cells could produce a factor or factors that alter 5 the expression in the kidney of accessory polypeptides required for mediating the effects of phosphatonin such as the phosphatonin receptor and intracellular proteins responsible for eliciting the effects of phosphatonin.

Altered gene expression by OOM tumor cells can also alter phosphate metabolism by more complex mechanisms. For example tumor produced factors could 10 up-regulate expression of genes normally controlled directly by phosphatonin or in response to phosphatonin. Such OOM tumor produced factors could increase expression of phosphate transport molecules and other cellular proteins necessary for regulating either phosphate uptake or secretion of phosphate. Alternatively, OOM tumor factors could alter expression of extracellular regulators or carriers of phosphate 15 or phosphatonin.

OOM-related genes that modulate phosphate metabolism are useful candidates for developing therapeutic agents for a variety of disease conditions related to abnormal phosphate metabolism. These include renal conditions such as renal osteodystrophy, changes in phosphate homeostasis after kidney transplant, end stage renal disease 20 (ESRD), and acute renal disease, bone defects, hypophosphataemia, hyperphosphataemia, hypoparathyroidism, and pseudohypoparathyroidism.

Phosphate metabolism related factors could provide useful mediators of disease conditions through a variety of alternative mechanisms. For example, during ESRD, phosphatonin or other proteins in its pathway may inhibit absorption of phosphate in the 25 small intestine. Such factors may also enhance phosphate uptake in the proximal tubules of the kidney. Modulation of the activity of these factors could therefore be used to control the symptoms of this disease.

In conditions characterized by hypophosphatemia or low serum phosphate levels blocking any protein that is involved in lowering serum phosphate levels or inhibiting 30 its functions could be an effective therapy. This type of therapy could be useful for a range of conditions including hyperparathyroidism, X-linked hypophosphatemic rickets, vitamin D dependent rickets, Franconi Syndrome, post kidney transplant condition, and oncogenic osteomalacia.

Diseases characterized by increased phosphate levels or hyperphosphatemia could be affected by treatment directed towards any protein that acts in the phosphatonin pathway to lower serum phosphate levels. Diseases related to hyperphosphatemia include: hypoparathyroidism (levels of PTH secreted are insufficient to maintain 5 extracellular calcium and phosphate levels-leads to hypocalcemia and hyperphosphatemia); pseudohypoparathyroidism (a group of disorders characterized by biochemical hypoparathyroidism, hypocalcemia and hyperphosphatemia, increased secretion of PTH and resistance to the biological actions of PTH); transcellular phosphate shift from cells into the extracellular fluid caused by systemic infections, 10 severe hyperthermia, crush injuries, non-traumatic rhabdomyolysis, and tumor lysis syndrome after cytotoxic therapies for hematologic malignancies; and renal disease.

In addition to modulation of phosphate metabolism, factors whose expression is altered in OOM tumor cells can include genes whose polypeptide products act directly 15 on osteogenic cells to mediate bone mineralization. Such proteins associated with OOM may either promote or inhibit diseases associated with defective mineralization. Possible functions of proteins in the bone mineralization pathway include: inhibition of bone mineralization, regulation of the early stages of bone mineralization, and control of bone cell differentiation and bone development.

A variety of types of polypeptide factors may be found to modulate bone 20 mineralization. For example extracellular matrix proteins (ECM) are an important constituent of bone. In bone, cartilage and the tissues forming the teeth, unlike those in other connective tissues, the matrices have the unique ability to become calcified. Furthermore control of cell viability and morphogenesis is well known to be affected by 25 appropriate contact with a wide array of ECM proteins. Thus OOM tumor produced ECM proteins could alter the natural process of bone mineral homeostasis by acting directly on bone cells. Alternatively, OOM tumor cells could produce diffusible soluble factors that regulate bone cell differentiation, growth and metabolism. Such factors also provide useful targets for development of therapeutic agents to regulate bone mineralization.

30 A number of serious pathological conditions are related to defects in bone mineralization. These include osteoporosis (a metabolic bone disease characterized by low bone mass and microarchitectural deterioration of bone tissue); osteomalacia (a defect in bone mineralization that occurs after the cessation of growth and involves only

the bone and not the growth plate); rickets (a disorder of mineralization of the bone matrix, or osteoid, in growing bones; that involves both the growth plate (epiphysis) and newly formed trabecular and cortical bone); hypophosphatasis (a rare heritable type of rickets or osteomalacia (1 in 100,000 births) characterized by a reduction of activity of 5 the tissue non-specific isoenzyme of alkaline phosphatase); and Fanconi syndrome and renal tubular acidosis (a generalized defect in renal proximal tubule transport capacity that includes impaired reabsorption of glucose, phosphate, amino acids, bicarbonate, uric acid, citrate and other organic acids, and low-molecular weight proteins and that is associated with rickets and osteomalacia).

10 OOM tumor produced factors that are found to modulate fundamental processes involved in bone formation, mineralization and maintenance could provide useful targets to inhibit the progression of these diseases.

15 In addition to diseases characterized by defects in bone mineralization, pathological conditions of the bone include defects in bone remodeling such as Paget's disease, osteomyelitis, osteosarcoma and stress fracture. As in the case of defective bone mineralization, polypeptide factors identified from OOM tumor cells that directly modulate bone metabolism and bone cell development are useful targets for developing novel therapeutic agents to treat diseases characterized by alternative bone pathologies. Furthermore, in certain cases, expression of OOM tumor associated factors may be 20 found to be diagnostic of bone disease making these genes useful markers for diagnostic tests to identify such conditions.

25 In one embodiment of the invention the oncogenic osteomalacia-related polynucleotide is isolated using the SAGE technique (Serial Analysis of Gene Expression or "SAGE," disclosed in Velculescu, et al. (1995) *Science* 270:484-487 and U.S. Patent No. 5,695,937). Using the SAGE tag for the polynucleotide of the present invention a full length cDNA encoding a oncogenic osteomalacia-related factor was isolated by hybridization to a cDNA library derived from an appropriate oncogenic osteomalacia cell. The additional embodiments of this invention can be isolated using the sequences provided in SEQ ID NOS:1-2, and the methods described below or by 30 homology searching publicly available databases

In addition to the sequence shown in SEQ ID NO:1, or their complements, this invention also provides the anti-sense polynucleotide strand, e.g. antisense RNA to these sequence or its complement. One can obtain an antisense RNA using the

sequence provided in SEQ ID NO:1, and the methodology described in Vander Krol, et al. (1988) *BioTechniques* 6:958.

Alternatively, biologically equivalent polynucleotides can be identified using sequence homology searches. Several embodiments of biologically equivalent

5 polynucleotides are within the scope of this invention, e.g., those characterized by possessing at least 75%, or at least 80%, or at least 90% or at least 95% sequence homology as determined using a sequence alignment program under default parameters correcting for ambiguities in the sequence data, changes in nucleotide sequence that do not alter the amino acid sequence because of degeneracy of the genetic code,

10 conservative amino acid substitutions and corresponding changes in nucleotide sequence, and variations in the lengths of the aligned sequences due to splicing variants or small deletions or insertions between sequences that do not affect function.

A variety of software programs are available in the art. Non-limiting examples of these programs are BLAST family programs including BLASTN, BLASTP,

15 BLASTX, TBLASTN, and TBLASTX (BLAST is available from the worldwide web at <http://www.ncbi.nlm.nih.gov/BLAST/>), FastA, Compare, DotPlot, BestFit, GAP, FrameAlign, ClustalW, and PileUp. These programs can be obtained commercially in a comprehensive package of sequence analysis software such as GCG Inc.'s Wisconsin Package. Other similar analysis and alignment programs can be purchased from various providers such as DNA Star's MegAlign, or the alignment programs in GeneJockey.

20 Alternatively, sequence analysis and alignment programs can be accessed through the world wide web at sites such as the CMS Molecular Biology Resource at <http://www.sdsc.edu/ResTools/crmshp.html>. Any sequence database that contains DNA or protein sequences corresponding to a gene or a segment thereof can be used for

25 sequence analysis. Commonly employed databases include but are not limited to GenBank, EMBL, DDBJ, PDB, SWISS-PROT, EST, STS, GSS, and HTGS. Sequence similarity can be discerned by aligning the tag sequence against a DNA sequence database. Alternatively, the tag sequence can be translated into six reading frames; the predicted peptide sequences of all possible reading frames are then compared to

30 individual sequences stored in a protein database such as s done using the BLASTX program.

Parameters for determining the extent of homology set forth by one or more of the aforementioned alignment programs are well established in the art. They include but

are not limited to p value, percent sequence identity and the percent sequence similarity.

P value is the probability that the alignment is produced by chance. For a single alignment, the p value can be calculated according to Karlin et al. (1990) *Proc. Natl. Acad. Sci. USA* 87: 2246.

For multiple alignments, the p value can be calculated using a

5 heuristic approach such as the one programmed in BLAST. Percent sequence identify is defined by the ratio of the number of nucleotide or amino acid matches between the query sequence and the known sequence when the two are optimally aligned. The percent sequence similarity is calculated in the same way as percent identity except one scores amino acids that are different but similar as positive when calculating the percent 10 similarity. Thus, conservative changes that occur frequently without altering function, such as a change from one basic amino acid to another or a change from one hydrophobic amino acid to another are scored as if they were identical. A tag sequence is considered to lack substantial homology with any known sequences when the regions of alignment of comparable length exhibit less than 30% of sequence identity, more 15 preferably less than 20% identity, even more preferably less than 10% identity.

The polynucleotides of the invention can comprise additional sequences, such as coding sequences within the same transcription unit, controlling elements such as promoters, ribosome binding sites, and polyadenylation sites, additional transcription units under control of the same or a different promoter, sequences that permit cloning, 20 expression, and transformation of a host cell, and any such construct as may be desirable to provide embodiments of this invention.

Indeed, this invention also provides a promoter sequence derived from a cell's genome, wherein the promoter sequence corresponds to the regulatory region of a gene that is differentially transcribed in a cell expressing oncogenic osteomalacia-related

25 polypeptide as compared to a cell that does not express oncogenic osteomalacia-related polypeptide. The promoters are identified and characterized by: 1) probing a cDNA library with a probe corresponding to a SAGE tag sequence or generating a portion of the desired cDNA by conducting anchored PCR using primers based on the SAGE tag sequence. The partial cDNA product obtained in step one above can be used as a probe 30 to clone the extreme 5' end of the cDNA. Then, by using the 5' end of the cDNA as a probe, the promoter of the gene that encodes the cDNA can be cloned from a genomic DNA library. Functionally equivalent promoter sequences, as defined above, are further provided by this invention.

The promoters identified above can be operatively linked to a foreign polynucleotide to compel differential transcription of the foreign polynucleotide in the cell from which the promoter was derived. Cells containing these sequences are termed genetically modified cells. In one embodiment, a foreign polynucleotide is any 5 sequence that encodes in whole or in part a polypeptide or protein. It also includes sequences encoding ribozymes and antisense molecules. It further includes regulatory sequences upstream from a gene corresponding to a polynucleotide of this invention.

Foreign polynucleotides also include therapeutic genes that encode dominant inhibitory oligonucleotides and peptides as well as genes that encode regulatory proteins 10 and oligonucleotides. Generally, gene therapy will involve the transfer of a single therapeutic gene although more than one gene may be necessary for the treatment of particular diseases. In one embodiment, the therapeutic gene is a dominant inhibiting mutant of the wild-type oncogenic osteomalacia-related protein as shown in the Figure. Alternatively, the therapeutic gene could be a wild-type copy of a defective gene or a 15 functional homolog.

The polynucleotides of the invention can be introduced by any suitable gene delivery method or vector. They also can be expressed in a suitable host cell for generating a cell-based therapy. These methods are described in more detail below.

This invention also provides genetically modified cells that produce enhanced 20 expression of oncogenic osteomalacia-related polypeptide as compared to wild-type cells. The genetically modified cells can be produced by insertion of upstream regulatory sequences such as promoters or gene activators (see, U.S. Patent No. 5,733,761).

The polynucleotides and sequences identified above can be conjugated to a 25 detectable marker, e.g., an enzymatic label or a radioisotope for detection of nucleic acid and/or expression of the gene in a cell. A wide variety of appropriate detectable markers are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one will likely desire to employ a fluorescent label or an 30 enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with

complementary nucleic acid-containing samples. Briefly, this invention further provides a method for detecting a single-stranded polynucleotide identified by SEQ ID NO:1, or their complements, by contacting target single-stranded polynucleotides with a labeled, single-stranded polynucleotide (a probe) which is a portion of the nucleotides shown in

5 SEQ ID NO:1 (or the corresponding complement) under conditions permitting hybridization (preferably moderately stringent hybridization conditions) of complementary single-stranded polynucleotides, or more preferably, under highly stringent hybridization conditions. Hybridized polynucleotide pairs are separated from un-hybridized, single-stranded polynucleotides. The hybridized polynucleotide pairs are

10 detected using methods well known to those of skill in the art and set forth, for example, in Sambrook, et al. (1989) *supra*.

The polynucleotides and sequences embodied in this invention can be obtained using chemical synthesis, recombinant cloning methods, PCR, or any combination thereof. Methods of chemical polynucleotide synthesis are well known in the art and

15 need not be described in detail herein. One of skill in the art can use the sequence data provided herein to obtain a desired polynucleotide by employing a DNA synthesizer or ordering from a commercial service.

Compositions containing a carrier and the polynucleotides and sequences of this invention, in isolated form or contained within a vector or host cell are further provided

20 herein. When these compositions are to be used pharmaceutically, they are combined with a pharmaceutically acceptable carrier.

Suitable cell or tissue samples used for this invention encompass body fluid, solid tissue samples, tissue cultures or cells derived therefrom and the progeny thereof, and sections or smears prepared from any of these sources, or any other samples that

25 may contain a neoplastic tumor tissue.

The polynucleotides of this invention can be isolated using the technique described in the experimental section or replicated using PCR. The PCR technology is the subject matter of U.S. Patent Nos. 4,683,195; 4,800,159; 4,754,065; and 4,683,202 and described in PCR: THE POLYMERASE CHAIN REACTION (Mullis et al. eds,

30 Birkhauser Press, Boston (1994)) or MacPherson, et al. (1991) and (1994), *supra*, and references cited therein. Alternatively, one of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to replicate the DNA. Accordingly, this invention also provides a process for obtaining the polynucleotides of this invention

by providing the linear sequence of the polynucleotide, nucleotides, appropriate primer molecules, chemicals such as enzymes and instructions for their replication and chemically replicating or linking the nucleotides in the proper orientation to obtain the polynucleotides. In a separate embodiment, these polynucleotides are further isolated.

5 Still further, one of skill in the art can insert the polynucleotide into a suitable replication vector and insert the vector into a suitable host cell (prokaryotic or eukaryotic) for replication and amplification. The DNA so amplified can be isolated from the cell by methods well known to those of skill in the art. A process for obtaining polynucleotides by this method is further provided herein as well as the polynucleotides 10 so obtained.

RNA can be obtained by first inserting a DNA polynucleotide into a suitable host cell. The DNA can be inserted by any appropriate method, e.g., by the use of an appropriate gene delivery vehicle (e.g., liposome, plasmid or vector) or by electroporation. When the cell replicates and the DNA is transcribed into RNA; the

15 RNA can then be isolated using methods well known to those of skill in the art, for example, as set forth in Sambrook, et al. (1989) *supra*. For instance, mRNA can be isolated using various lytic enzymes or chemical solutions according to the procedures set forth in Sambrook, et al. (1989), *supra* or extracted by nucleic-acid-binding resins following the accompanying instructions provided by manufacturers.

20 Polynucleotides exhibiting sequence complementarity or homology to SEQ ID NO:1 find utility as hybridization probes. Since the full coding sequence of the transcript is known, any portion of this sequence or homologous sequences, can be used in the methods of this invention.

It is known in the art that a "perfectly matched" probe is not needed for a 25 specific hybridization. Minor changes in probe sequence achieved by substitution, deletion or insertion of a small number of bases do not affect the hybridization specificity. In general, as much as 20% base-pair mismatch (when optimally aligned) can be tolerated. Preferably, a probe useful for detecting the aforementioned mRNA is at least about 80% identical to the homologous region. More preferably, the probe is 30 85% identical to the corresponding gene sequence after alignment of the homologous region; even more preferably, it exhibits 90% identity.

These probes can be used in radioassays (e.g. Southern and Northern blot analysis) to detect, prognose, diagnose or monitor various neoplastic cells or tumor

tissues containing these cells. The probes also can be attached to a solid support or an array such as a chip for use in high throughput screening assays for the detection of expression of the gene corresponding a polynucleotide of this invention. Accordingly, this invention also provides a probe comprising or corresponding to a polynucleotide of 5 SEQ ID NO:1, or its complement, or a fragment of SEQ ID NO:1, attached to a solid support for use in high throughput screens.

The total size of fragment, as well as the size of the complementary stretches, will depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the 10 length of the complementary region may be varied, such as between at least 5 to 10 to about 100 nucleotides, or even full length according to the complementary sequences one wishes to detect.

Nucleotide probes having complementary sequences over stretches greater than 5 to 10 nucleotides in length are generally preferred, so as to increase stability and 15 selectivity of the hybrid, and thereby improving the specificity of particular hybrid molecules obtained. More preferably, one can design polynucleotides having gene-complementary stretches of 10 or more or more than 50 nucleotides in length, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid 20 reproduction technology, such as the PCR technology with two priming oligonucleotides as described in U.S. Patent No. 4,603,102 or by introducing selected sequences into recombinant vectors for recombinant production. A preferred probe is about 50-75 or more preferably, 50-100, nucleotides in length.

The polynucleotides of the present invention can serve as primers for the 25 detection of genes or gene transcripts that are expressed in neoplastic cells associated with oncogenic osteomalacia. In this context, amplification means any method employing a primer-dependent polymerase capable of replicating a target sequence with reasonable fidelity. Amplification may be carried out by natural or recombinant DNA-polymerases such as T7 DNA polymerase, Klenow fragment of *E. coli* DNA 30 polymerase, and reverse transcriptase. A preferred length of the primer is the same as that identified for probes, above.

A preferred amplification method is PCR. However, PCR conditions used for each reaction are empirically determined. A number of parameters influence the success

of a reaction. Among them are annealing temperature and time, extension time, Mg<sup>2+</sup> concentration, pH, and the relative concentration of primers, templates, and deoxyribonucleotides. After amplification, the resulting DNA fragments can be detected by agarose gel electrophoresis followed by visualization with ethidium bromide staining and ultraviolet illumination.

The invention further provides the isolated polynucleotide operatively linked to a promoter of RNA transcription, as well as other regulatory sequences for replication and/or transient or stable expression of the DNA or RNA. As used herein, the term "operatively linked" means positioned in such a manner that the promoter will direct transcription of RNA off the DNA molecule. Examples of such promoters are SP6, T4 and T7. In certain embodiments, cell-specific promoters are used for cell-specific expression of the inserted polynucleotide. Vectors which contain a promoter or a promoter/enhancer, with termination codons and selectable marker sequences, as well as a cloning site into which an inserted piece of DNA can be operatively linked to that promoter are well known in the art and commercially available. For general methodology and cloning strategies, see GENE EXPRESSION TECHNOLOGY (Goeddel ed., Academic Press, Inc. (1991)) and references cited therein and VECTORS: ESSENTIAL DATA SERIES (Gacesa and Ramji, eds., John Wiley & Sons, N.Y. (1994)), which contains maps, functional properties, commercial suppliers and a reference to GenEMBL accession numbers for various suitable vectors. Preferable, these vectors are capable of transcribing RNA *in vitro* or *in vivo*.

In one embodiment, polynucleotides derived from the polynucleotides of the invention encode polypeptides or proteins having diagnostic and therapeutic utilities as described herein as well as probes to identify transcripts of the protein that may or may not be present. These nucleic acid fragments can be prepared, for example, by restriction enzyme digestion of the larger polynucleotides such as SEQ ID NO:1 or its complement, and then labeled with a detectable marker. Alternatively, random fragments can be generated using nick translation of the molecule. For methodology for the preparation and labeling of such fragments, see Sambrook, et al., (1989) *supra*.

Expression vectors containing these nucleic acids are useful to obtain host vector systems to produce proteins and polypeptides. It is implied that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include plasmids. A preferred length

Compositions containing the polynucleotides of this invention, in isolated form or contained within a vector or host cell are further provided herein. When these compositions are to be used pharmaceutically, they are combined with a pharmaceutically acceptable carrier.

5 In one embodiment of the invention, the information obtained on a gene of interest that is identified using methods described herein is used to create "knock-out" animals, in which the gene of interest is deleted or mutated sufficiently to disrupt its function. The resulting transgenic animals can be used to analyze the function of the gene. These "knock-out" animals are made by taking advantage of the phenomena of  
10 homologous recombination, using methods well known in the art. Briefly, targeting DNA vectors contain (1) two blocks of DNA sequences that are homologous to separate regions of the target site; (2) a DNA sequence that codes for resistance to the compound G418 (Neo') between the two blocks of homologous DNA (*i.e.* positive selection marker) and (3) DNA sequences coding for herpes simplex virus thymidine kinases  
15 (HSV-tk1 and HSV-tk2) outside of the homologous blocks (*i.e.* negative selection marker). When this vector is introduced into the embryonic stem cell, homologous recombination inserts the Neo' gene into the target genome, disrupting function of that gene.

20 **Proteins**

This invention provides proteins or polypeptides expressed from the polynucleotides of this invention, which is intended to include wild-type and recombinantly produced polypeptides and proteins from prokaryotic and eukaryotic host cells, as well as muteins, analogs and fragments thereof. In some embodiments, the  
25 term also includes antibodies and anti-idiotypic antibodies. In one embodiment, these proteins or polypeptides are a oncogenic osteomalacia-related factor, which modulates phosphatonin activity. Such polypeptides can be isolated or produced using the methods identified below.

It is understood that functional equivalents or variants of the wild-type  
30 polypeptide or protein also are within the scope of this invention, for example, those having conservative amino acid substitutions of the sequence shown in SEQ ID NO:2 from amino acid 1 to 525 or from amino acid 17 to 525. Other analogs include fusion proteins comprising a protein or polypeptide.

The proteins and polypeptides of this invention are obtainable by a number of processes well known to those of skill in the art, which include purification, chemical synthesis and recombinant methods. Full length proteins can be purified from a neoplastic cell or a tumor biopsy as identified above. Sources for purifying the protein 5 can also be serum or urine samples from an individual, such as a patient with oncogenic osteomalacia. Proteins can be purified by methods such as immunoprecipitation with antibody, and standard techniques such as gel filtration, ion-exchange, reversed-phase, and affinity chromatography using a fusion protein as shown herein. For such methodology, see for example Deutscher et al. (1999) GUIDE TO PROTEIN PURIFICATION: 10 METHODS IN ENZYMOLOGY (Vol. 182, Academic Press). Accordingly, this invention also provides the processes for obtaining these proteins and polypeptides as well as the products obtainable and obtained by these processes.

The proteins and polypeptides also can be obtained by chemical synthesis using a commercially available automated peptide synthesizer such as those manufactured by 15 Perkin Elmer/Applied BioSystems, Inc., Model 430A or 431A, Foster City, CA, USA. The synthesized protein or polypeptide can be precipitated and further purified, for example by high performance liquid chromatography (HPLC). Accordingly, this invention also provides a process for chemically synthesizing the proteins of this invention by providing the sequence of the protein and reagents, such as amino acids 20 and enzymes and linking together the amino acids in the proper orientation and linear sequence.

Alternatively, the proteins and polypeptides can be obtained by well-known recombinant methods as described, for example, in Sambrook et al. (1989), *supra*, using the host cell and vector systems described above.

25 Also provided by this application are the polypeptides and proteins described herein conjugated to a detectable agent for use in the diagnostic methods. For example, detectably labeled proteins and polypeptides can be bound to a column and used for the detection and purification of antibodies. They also are useful as immunogens for the production of antibodies as described below. The proteins and fragments of this 30 invention are useful in an *in vitro* assay system to screen for agents or drugs, which modulate cellular processes.

The proteins of this invention also can be combined with various liquid phase carriers, such as sterile or aqueous solutions, pharmaceutically acceptable carriers,

suspensions and emulsions. Examples of non-aqueous solvents include propyl ethylene glycol, polyethylene glycol and vegetable oils. When used to prepare antibodies, the carriers also can include an adjuvant that is useful to non-specifically augment a specific immune response. A skilled artisan can easily determine whether an adjuvant is required and select one. However, for the purpose of illustration only, suitable adjuvants include, but are not limited to Freund's Complete and Incomplete, mineral salts and polynucleotides.

10 This invention also provides a pharmaceutical composition comprising any of a protein, analog, mutein, polypeptide fragment, antibody, antibody fragment or anti-idiotopic antibody of this invention, alone or in combination with each other or other agents, and an acceptable carrier. These compositions are useful for various diagnostic and therapeutic methods as described herein.

### Antibodies

15 Also provided by this invention is an antibody capable of specifically forming a complex with the proteins or polypeptides as described above. The term "antibody" includes polyclonal antibodies and monoclonal antibodies. The antibodies include, but are not limited to mouse, rat, and rabbit or human antibodies.

20 Laboratory methods for producing polyclonal antibodies and monoclonal antibodies, as well as deducing their corresponding nucleic acid sequences, are known in the art, see Harlow and Lane (1988) *supra* and Sambrook, et al. (1989) *supra*. The monoclonal antibodies of this invention can be biologically produced by introducing protein or a fragment thereof into an animal, e.g., a mouse or a rabbit. The antibody producing cells in the animal are isolated and fused with myeloma cells or 25 heteromyeloma cells to produce hybrid cells or hybridomas. Accordingly, the hybridoma cells producing the monoclonal antibodies of this invention also are provided.

30 Thus, using the protein or fragment thereof, and well known methods, one of skill in the art can produce and screen the hybridoma cells and antibodies of this invention for antibodies having the ability to bind the proteins or polypeptides.

If a monoclonal antibody being tested binds with the protein or polypeptide, then the antibody being tested and the antibodies provided by the hybridomas of this invention are equivalent. It also is possible to determine without undue

experimentation, whether an antibody has the same specificity as the monoclonal antibody of this invention by determining whether the antibody being tested prevents a monoclonal antibody of this invention from binding the protein or polypeptide with which the monoclonal antibody is normally reactive. If the antibody being tested 5 competes with the monoclonal antibody of the invention as shown by a decrease in binding by the monoclonal antibody of this invention, then it is likely that the two antibodies bind to the same or a closely related epitope. Alternatively, one can pre-incubate the monoclonal antibody of this invention with a protein with which it is normally reactive, and determine if the monoclonal antibody being tested is inhibited in 10 its ability to bind the antigen. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or a closely related, epitopic specificity as the monoclonal antibody of this invention.

The term "antibody" also is intended to include antibodies of all isotypes. Particular isotypes of a monoclonal antibody can be prepared either directly by selecting 15 from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:8653 or Spira et al. (1984) *J. Immunol. Meth.* 74:307.

This invention also provides biological active fragments of the polyclonal and 20 monoclonal antibodies described above. These "antibody fragments" retain some ability to selectively bind with its antigen or immunogen. Such antibody fragments can include, but are not limited to: Fab; Fab'; F(ab')<sub>2</sub>; Fv; and SCA.

A specific example of "a biologically active antibody fragment" is a CDR region 25 of the antibody. Methods of making these fragments are known in the art, see for example, Harlow and Lane, (1988) *supra*.

The antibodies of this invention also can be modified to create chimeric antibodies and humanized antibodies (Oi et al. (1986) *BioTechniques* 4(3):214). Chimeric antibodies are those in which the various domains of the antibodies' heavy and light chains are coded for by DNA from more than one species.

30 The isolation of other hybridomas secreting monoclonal antibodies with the specificity of the monoclonal antibodies of the invention can also be accomplished by one of ordinary skill in the art by producing anti-idiotypic antibodies (Herlyn et al. (1986) *Science* 232:100). An anti-idiotypic antibody is an antibody that recognizes

unique determinants present on the monoclonal antibody produced by the hybridoma of interest.

Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their 5 recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody it is possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies that mimic an epitope. For example, an anti-idiotypic monoclonal antibody 10 made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the mirror image of the epitope bound by the first monoclonal antibody. Thus, in this instance, the anti-idiotypic monoclonal antibody could be used for immunization for production of these antibodies.

As used in this invention, the term "epitope" is meant to include any determinant 15 having specific affinity for the monoclonal antibodies of the invention. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

The antibodies of this invention can be linked to a detectable agent or label. 20 There are many different labels and methods of labeling known to those of ordinary skill in the art.

The antibody-label complex is useful to detect the protein or fragments in a sample, using standard immunochemical techniques such as immunohistochemistry as described by Harlow and Lane (1988) *supra*. Competitive and non-competitive 25 immunoassays in either a direct or indirect format are examples of such assays, e.g., enzyme linked immunoassay (ELISA) radioimmunoassay (RIA) and the sandwich (immunometric) assay. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The coupling of antibodies to low molecular weight haptens can increase the 30 sensitivity of the assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts avidin, or dinitrophenyl, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies. See Harlow and Lane (1988) *supra*.

The monoclonal antibodies of the invention also can be bound to many different carriers. Thus, this invention also provides compositions containing the antibodies and another substance, active or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and 5 modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

10 Compositions containing the antibodies, fragments thereof or cell lines which produce the antibodies, are encompassed by this invention. When these compositions are to be used pharmaceutically, they are combined with a pharmaceutically acceptable carrier.

#### **Functional analysis**

15 The ability of factors identified by the present invention to modulate phosphatonin activity, to participate in phosphate metabolism or to modulate bone mineralization can be demonstrated using various methods commonly employed by practitioners of the art.

20 To perform functional analysis a full length copy of a cDNA corresponding to the expressed Oncogenic Osteomalacia Related Protein 1 gene (OOM1) was isolated as follows. The 15 nucleotide SAGE tag sequence corresponding to OOM 1 was extended to 26 nucleotides using anchored PCR. The resulting product sequence, "cat gaa aat aaa 25 caa tat ttc tc" (SEQ ID NO:3) was used to probe a lambda phage library derived from RNA isolated from two OOM tumors to isolate full length cDNA's corresponding to the OOM1 SAGE tag.

#### **Analysis of function by bioinformatics**

30 Bioinformatics analysis of the sequences of the polynucleotides and polypeptides of the invention can be performed to identify key structural features and to determine specific biochemical properties and activities of these compositions. For example, in many cases it is possible to infer the functions of novel genes through comparisons with the functions of known homologies of these genes that are identified using sequence analysis software. In particular, many proteins share variant forms of known functional

domains, which have been well characterized for their biological activities. These homologies can be identified by basic alignment software tools such as BLAST programs or by more sophisticated approaches such as Profile analysis to detect functional protein motifs with various degrees of sequence homology. For example, one 5 can identify protein domains such as sarc homology domains known to be involved in signal transduction, helix-turn-helix, leucine zipper, or zinc finger motifs found in transcription factors, motifs like immunoglobulin domains, EGF domains, or fibronectin III domains, common to extracellular proteins such as cell surface receptors, or many other well characterized protein domains well known to those practiced in the art. It is 10 also possible to deduce function by identification of homology with novel proteins found in various model experimental organisms such as yeast, *C. elegans*, or *Drosophila*. In these cases, experimental evaluation of orthologous genes in the model organism can be used to predict the function of the identified mammalian genes.

Computer analysis of the predicted polypeptide sequence encoded by the OOM1 15 cDNA identified a number of sequence motifs that provide useful predictions about its function. Analysis of the primary amino acid sequence indicates that the protein secondary structure contains 16% alpha-helix, 8% beta-sheet, 76% random coil. The overall structure of the OOM1 protein contains a small acidic ordered structure, followed by a random unfolded structure, and a third domain consisting of a more 20 ordered structure (somewhat like a dumbbell). Comparison with protein structure databases indicates that this general structure can be found in proteins that function as transcription factors, extracellular matrix proteins, or prepro-forms of peptide factors.

Protein motif analysis further indicates that OOM1 contains the following 25 protein motifs (Figure 2): Signal peptide (67% probability), a nuclear localization signal (assume signal peptide has priority over the NLS), 2 potential glycosylation sites, 1 potential cAMP dependent PK phosphorylation site, 16 potential protein kinase C phosphorylation sites, 1 tyrosine kinase phosphorylation site, an RGD domain for cell attachment, a calcium binding domain similar to that found in calcium binding protein and porin:

30

Calcium Binding Protein	GIPDYQEDEDSDGIPDYLD (SEQ ID NO:4)
Porin	GYTDLDDDRGGNDIPYLTGD (SEQ ID NO:5)
OOM1	GYPDQLQERGDNDISPFSGD (SEQ ID NO:6)

A sequence within this putative calcium binding domain is similar to a calcium binding motif found in N-CAM

5	N-CAM	DLQERGDSD (SEQ ID NO:7)
	OOM1	DLQERGDND (SEQ ID NO:8)

Based on this structure analysis the following protein types have been ruled out: enzymes (including kinases and phosphatases), membrane proteins, and certain extracellular matrix proteins such as collagens.

10 Blast searches of the complete protein sequence reveal no significant homologies. However limited overall homologies include: a hypothetical protein from *Plasmodium falciparum*, a malaria parasite-infected erythrocyte antigen, and a natural killer cell tumor-recognition protein.

15 Blast searches to partial segments of the protein reveals that the acidic domain shares homologies with specific regions within the following genes: intern binding bone sialoprotein I (osteopontin), drosophila "disabled" product homology, Na/CaK exchanger, drosophila "hedgehog" protein homologue, collagen, neural precursor cell expressed developmentally down-regulated Nedd 9, neurofilament medium protein, and myosin phosphatase target subunit.

20 Although the structure and presence of a nuclear localization signal suggest a relationship with transcription factors, the presence of a signal peptide (and no membrane spanning domain) indicates that this protein is secreted and therefore not involved in transcription. The further presence of calcium binding motifs found in extracellular proteins and a cell attachment RGD motif further indicate that the OOM1 25 protein functions as an extracellular molecule.

The overall structure of the encoded protein (ordered structure, elongated structure followed by an ordered structure) is consistent with certain secreted proteins involved in regulation of hormone or peptide signaling such as hedgehog and frizzled related protein, prepro-proteins of peptide factors, or extracellular matrix proteins.

30

#### Protein function assays

Limited data measuring the phosphatoin-related activity of OOM1 indicates the OOM1 encoded protein does not have an effect on phosphatoin. This data does not address whether phosphatoin activity may be controlled by either a modified form of

OOM1 (for example, a proteolytic fragment, calcium binding, phosphorylation, etc) and/or through the actions of accessory proteins. Thus OOM1 may be involved in modulating phosphatonin activity.

### 5 Expression analysis

The activities of the polynucleotides of this invention can be further analyzed by measuring their expression in various cells involved in phosphate metabolism. Results of this analysis can then be compared with the expression pattern that is observed in alternative tissues and cells samples. Methods that can be used for such analysis include

10 the SAGE technique, RT-PCR, Northern blots, Rnase protection assays, DNA microarrays, and in situ hybridization.

Analysis of OOM1 gene expression by in situ hybridization indicates that the gene is highly expressed in murine embryos. In addition, the expression during embryonic development suggests that the OOM1 protein participates in morphogenesis

15 or cellular differentiation.

Structural features of the OOM1 protein are consistent with an activity involving the extracellular matrix. The protein contains a secretion signal, it has a general structure similar to known ECM proteins, and it has an RGD motif well known to mediate attachment to certain integrin molecules.

20 OOM1 expression has been observed in chondrocytes and osteoblasts in mouse embryos using in situ hybridization techniques, consistent with the interpretation that the OOM1 protein has a function related to the differentiation and/or regulation of cartilage and bone. In addition in situ RNA staining is observed in osteoblasts within embryos and OOM tumors. These observations are consistent with a role for the OOM1

25 protein in controlling differentiation of cells or modulating bone mineralization. Finally in situ RNA hybridization staining is observed in normal epithelial cells. The implications of this result for OOM1 function have not been determined.

### Gain of function and loss of function experiments

30 Similar demonstrations of gene function can also be performed using expression vectors that comprise the polynucleotides of the invention, by transfecting test cells in vitro with these expression vectors and measuring cell activities characteristic of phosphate metabolism such as phosphate transport. Alternatively, viral vectors

expressing these polynucleotides can be inserted into cells *in vitro* or *in vivo* to demonstrate function in modulating bone mineralization or phosphate metabolism, for example, by adding the vector to an *in vitro* bone cell differentiation assay or to an *in vivo* phosphate uptake assay. Also transgenic animals can be produced that overexpress 5 the identified genes to demonstrate their function.

In addition to such gain of function experiments, one can also perform loss of function experiments with the identified genes using alternative gene delivery systems that shut down expression of the oncogenic osteomalacia-related gene. This can be done with antisense oligonucleotides, antisense RNA or ribozyme vectors. One can also 10 produce transgenic knockout mice to analyze loss of function of the identified genes.

### Functional Analysis with Antibodies

Antibodies of this invention can be used to purify the polypeptides of this invention and to identify biological equivalent polynucleotides. They also can be used 15 to identify agents that modify the function of the polynucleotides of this invention. These antibodies include polyclonal antisera, monoclonal antibodies, and various reagents derived from these preparations that are familiar to those practiced in the art and described above. Antibodies can be used in immuno-histochemistry to determine the physical location of the proteins encoded by the identified genes in subjects with 20 abnormal phosphate metabolism such as in oncogenic osteomalacia and with model cells performing phosphate uptake. For example, physical association of such proteins with kidney cell surfaces or in the cytoplasm of kidney cells controlling phosphate uptake can be analyzed.

Antibodies that neutralize the activities of proteins encoded by identified genes 25 can also be used *in vivo* and *in vitro* to demonstrate function by adding such neutralizing antibodies into *in vivo* and *in vitro* test systems. They also are useful as pharmaceutical agents to modulate the activity of polypeptides of the invention.

Various antibody preparations can also be used in analytical methods such as 30 ELISA assays or Western blots to demonstrate the expression of proteins encoded by the identified genes by test cells *in vitro* or *in vivo*. Fragments of such proteins generated by protease degradation during metabolism can also be identified by using appropriate polyclonal antisera with samples derived from experimental samples.

### Screening Assays

The present invention provides methods for screening various agents that modulate the expression of a polynucleotide of the invention or the function of a protein product encoded by the polynucleotide of interest in a neoplastic cell associated with oncogenic osteomalacia. For the purposes of this invention, an "agent" is intended to include, but not be limited to a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide, a protein (e.g. antibody), a polynucleotide (e.g. anti-sense) or a ribozyme. A vast array of compounds can be synthesized, for example polymers, such as polypeptides and polynucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term "agent." In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. It should be understood, although not always explicitly stated that the agent is used alone or in combination with another agent, having the same or different biological activity as the agents identified by the inventive screen.

One preferred embodiment is a method for screening small molecules capable of interacting with the protein or polypeptide of the invention including, for example, oncogenic osteomalacia-related polypeptide produced from a neoplastic cell associated with oncogenic osteomalacia. For the purpose of this invention, "small molecules" are molecules having low molecular weights (MW) that are, in one embodiment, capable of binding to a protein of interest such as oncogenic osteomalacia-related polypeptide, and thereby altering the function of the protein. Preferably, the MW of a small molecule is no more than 1,000. Methods for screening small molecules capable of altering protein function are known in the art. For example, a miniaturized arrayed assay for detecting small molecule-protein interactions in cells is discussed by You et al. (1997) *Chem. Biol.* 4:961-968.

To practice the screening method *in vitro*, suitable cell cultures or tissue cultures containing this type of neoplastic cell are first provided. The cell can be a cultured cell or a genetically modified cell in which a transcript from SEQ ID NO:1, or its complement is expressed. Alternatively, the cells can be from a tissue biopsy. The cells are cultured under conditions (temperature, growth or culture medium and gas (CO<sub>2</sub>)) and for an appropriate amount of time to attain exponential proliferation without density

dependent constraints. It also is desirable to maintain an additional separate cell culture; one which does not receive the agent being tested as a control.

As is apparent to one of skill in the art, suitable cells may be cultured in microtiter plates and several agents may be assayed at the same time by noting 5 genotypic changes, phenotypic changes or cell death.

When the agent is a composition other than a DNA or RNA, such as a small molecule as described above, the agent may be directly added to the cell culture or added to culture medium for addition. As is apparent to those skilled in the art, an "effective" amount must be added which can be empirically determined. When the 10 agent is a polynucleotide, it may be directly added by use of a gene gun or electroporation. Alternatively, it may be inserted into the cell using a gene delivery vehicle or other method as described above.

Kits containing the agents and instructions necessary to perform the screen and 15 *in vitro* method as described herein also are claimed.

The assays also can be performed in a subject. When the subject is an animal such as a rat, mouse or simian, the method provides a convenient animal model system that can be used prior to clinical testing of an agent. In this system, a candidate agent is a potential drug if transcript expression is altered, i.e., upregulated (such as restoring 20 tumor suppressor function), downregulated or eliminated as with drug resistant genes or oncogenes, or if symptoms associated or correlated to the presence of cells containing transcript expression are ameliorated, each as compared to untreated, animal having the pathological cells. It also can be useful to have a separate negative control group of 25 cells or animals that are healthy and not treated, which provides a basis for comparison. After administration of the agent to subject, suitable cells or tissue samples are collected and assayed for altered gene expression or protein function.

Additionally, because Applicants have found that activity of the gene of this invention may be dependent on post-translational modification by serine protease 11, each of the above methods can be modified by delivering an effective amount of serine 30 protease 11 or by delivering an agent that up or down regulates expression of constitutive serine protease 11 expression. The serine protease 11 can be delivered as a polypeptide or as a polynucleotide encoding serine protease 11. The Genbank Accession number for serine protease 11 is AF07055 and is described by Zumbrunn and 35 Trueb (1996) *FEBS Lett.* **398**:187-192.

### Therapeutic Applications

The present invention encompasses methods of treating hypophosphataemia or hyperphosphataemia related diseases, such as oncogenic osteomalacia. The 5 polynucleotides or polypeptides of this invention and the above-described agents and their derivatives can be used, either alone or in conjunction with other active agents, in a pharmaceutical composition for the therapeutic treatments described herein.

In one preferred embodiment, a pharmaceutical composition comprising an 10 agent identified by the above-described screening assay is administered to a subject in an effective amount to treat hypophosphataemia related diseases, such as oncogenic osteomalacia, or to ameliorate the symptoms associated therewith. Preferably, the pharmaceutical composition is capable of modulating protein function in a subject with oncogenic osteomalacia; and thereby restoring normal serum phosphate levels in the 15 subject.

15 In another preferred embodiment, a pharmaceutical composition comprising a polypeptide of the invention is administered to a subject in an effective amount to treat hyperphosphataemia related diseases. Preferably, the pharmaceutical composition contains oncogenic osteomalacia-related polypeptide, a secreted protein, and is capable of lower the abnormally elevated serum phosphate levels in patients with 20 hyperphosphataemia related diseases. More preferably the oncogenic osteomalacia-related polypeptide-containing pharmaceutical composition further comprises active agents that promote the desired function in regulating phosphate homeostasis. Suitable active agents include, but are not limited to, enzymes or co-factors that are involved in the post-translational modification and processing of the mature oncogenic 25 osteomalacia-related protein; or factors responsible for maintaining the activated form of oncogenic osteomalacia-related polypeptide in circulation and at the site of phosphate homeostasis.

30 Various delivery systems are known and can be used to administer a therapeutic agent, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu (1987) *J. Biol. Chem.* 262:4429-4432), construction of a therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of delivery include but are not limited to transdermally, gene therapy, intra-arterial, intra-muscular, intravenous, intranasal, and oral routes, and

5 include sustained delivery systems. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, by injection, or by means of a catheter or targeted gene delivery of the sequence coding for the therapeutic.

The pharmaceutical compositions identified herein as effective for their intended purpose can be administered to subjects or individuals susceptible to or at risk of developing diseases associated with abnormal phosphate transport at kidney. When the agent is administered to a subject such as a mouse, a rat or a human patient, the agent 10 can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject. Therapeutic amounts can be empirically determined and will vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the agent.

15 Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple 20 administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be found below.

25 The agents and compositions of the present invention can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

The pharmaceutical compositions can be administered orally, intranasally, parenterally, transdermally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may 30 also take the form of gene therapy, suspensions, solutions and emulsions of the active ingredient in aqueous or nonaqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds of the invention.

Additionally, because Applicants have found that activity of the gene of this invention may be dependent on post-translational modification by serine protease 11, each of the above methods can be modified by delivering an effective amount of serine protease 11 or by delivering an agent that up or down regulates expression of 5 constitutive serine protease 11 expression. The serine protease 11 can be delivered as a polypeptide or as a polynucleotide encoding serine protease 11. The Genbank Accession number for serine protease 11 is AF07055 and is described by Zumbrunn and Trueb (1996) *FEBS Lett.* **398**:187-192.

It should be understood that in addition to the ingredients particularly mentioned 10 above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents. It also is intended that the agents, compositions and methods of this invention be combined with other suitable compositions and therapies.

15

#### **Identification, Analysis, and Manipulation of Genetic Polymorphisms with SNP Technology**

The isolated oncogenic osteomalacia-related gene can be used to search for and identify single nucleotide polymorphisms (SNP's), which are mutant variants of the 20 gene in the human population. Identification of such polymorphisms is useful to define human diseases to which mutations in the oncogenic osteomalacia-related gene contribute and to perfect therapies for disease processes in which the protein encoded by the oncogenic osteomalacia-related gene participates. Mutant variants of the gene identified in this manner can then be employed in the development, screening, and 25 analysis of pharmaceutical agents to treat these diseases. Methods to detect such SNP's can be formatted to create diagnostic tests. Furthermore, various mutations in the gene which effect the response of different individuals to therapeutic agents can be identified and then diagnosed through analysis of SNP's, to guide the prescription of appropriate treatments. Also, SNP's identified in the oncogenic osteomalacia-related gene can 30 provide useful sequence markers for genetic tests to analyze other genes and mutations in the region of the genome where the oncogenic osteomalacia-related gene is located. Thus it is useful to incorporate these SNP's into polymorphism databases.

Skilled practitioners of the art are familiar with an array of methods for

identifying and analyzing SNP's. High throughput DNA sequencing procedures such as sequencing by hybridization (Drmanac et al. (1993) *Science* **260**:1649-1652), minisequencing primer extension (Syvanen, (1999) *Hum. Mutat.* **13**(1):1-10), or other sequencing methods can be used to detect SNP's in defined regions of the gene.

5 Alternatively, hybridization to oligonucleotides on DNA microarrays (Lipshutz et al. (1999) *Nat. Genet.* **21**(1 Suppl.):20-24) analysis of single strand conformational polymorphisms in DNA or RNA molecules by various analytical methods (Nataraj (1999) *Electrophoresis* **20**(6):1177-85), PCR-based mutational analyses such as PCR with primers spanning the polymorphic sequence, or protection of SNP-containing 10 oligonucleotides from nuclease protection such as by use of the bacterial mutS protein can be employed. Many sophisticated high-throughput technologies based on methods such as automated capillary electrophoresis (Larsen et al. (1999) *Hum. Mutat.* **13**(4):318-327), time-of-flight mass spectroscopy (Li et al. (1999) *supra*, high density micro-arrays (Sapolsky et al. (1999) *Genet. Anal.* **14**(5-6):187-92), semiconductor 15 microchips (Gilles et al. (1999) *Nat. Biotechnol.* **17**(4):365-70), and others have been demonstrated that can be employed with the oncogenic osteomalacia-related gene to perform the uses described above.

### Genomics Applications

20 This invention also provides a process for preparing a database for the analysis of a cell's expressed genes by storing in a digital storage medium information related to the sequences of the transcriptome. Using this method, a data processing system for standardized representation of the expressed genes of a cell is compiled. In one embodiment, the database contains at least one polynucleotide of this invention. In 25 alternative embodiments, the database contains any combination of polynucleotides of this invention, alone or in combination with other polynucleotides or tags. The data processing system is useful to characterize the genotype or phenotype of a cell and to analyze gene expression between two cells by first selecting a cell and then identifying and sequencing the transcriptome of the cell. This information also is stored in a 30 computer-readable storage medium as the transcriptome. The transcriptome is then compared with at least one sequence(s) of transcription fragments from a polynucleotide of this invention. The compared sequences are then analyzed. Uniquely expressed

sequences and sequences differentially expressed between the hemangioma or endothelial cell and the selected cell can be identified by this method.

In other words, this invention provides a computer based method for screening the homology of an unknown DNA or mRNA sequence against one or more of transcribed or expressed genes of a preselected cell by first providing the complete set of expressed genes, i.e., the transcriptome, in computer readable form and homology screening the DNA or mRNA of the unknown sequence against transcriptome and determining whether the DNA sequence of the unknown contains similarities to any portion of the transcriptome listed in the computer readable form. In one embodiment, SEQ ID NO: 1 or polynucleotides corresponding to this sequence are the transcriptome against which test cells are compared.

Thus, the information provided herein also provides a means to compare the relative abundance of gene transcripts in different biological specimens by use of high-throughput sequence-specific analysis of individual RNAs or their corresponding cDNAs using a modification of the systems described in WO 95/2068, WO 96/23078 and U.S. Patent No. 5,618,672.

Additional utilities of the database include, but are not limited to analysis of the developmental state of a test cell, the influence of viral or bacterial infection, control of cell cycle, effect of a tumor suppressor gene or lack thereof, polymorphism within the cell type, apoptosis, and the effect of regulatory genes.

#### Non-Human Transgenic Animals

In another aspect, the novel polynucleotide sequences associated with a pathological state of a cell can be used to generate transgenic animal models. In recent years, geneticists have succeeded in creating transgenic animals, for example mice, by manipulating the genes of developing embryos and introducing foreign genes into these embryos. Once these genes have integrated into the genome of the recipient embryo, the resulting embryos or adult animals can be analyzed to determine the function of the gene. The mutant animals are produced to understand the function of known genes *in vivo* and to create animal models of human diseases. See, e.g., Chisaka et al. (1992) 355:516-520; Joyner et al. (1992) in POSTIMPLANTATION DEVELOPMENT IN THE MOUSE (Chadwick and Marsh, eds., John Wiley & Sons, United Kingdom) pp:277-297; Dorin et al. (1992) *Nature* 359:211-215.

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The following examples are intended to illustrate, but not limit this invention.

### Functional Analysis of the OOM-Related 1 Gene

A full length cDNAs encoding the OOM-related 1 protein was inserted into an expression vector and the OOM-Related 1 protein was expressed in mammalian culture cells using standard cloning strategies. Stable cell lines that can be rapidly scaled for production were also established. *In vitro* expressed OOM-related 1 was then produced as a secreted molecule in conditioned culture medium and prepared for functional assays measuring phosphate re-absorption.

Similar conditioned media were also prepared from mammalian cells that had been transfected with a control vector that did not contain any recombinant gene as well as mammalian cells that had been transfected with the serine protease 11 gene (GenBank accession # AF070555).

15 **Phosphate transport assay**

The phosphate transport modulating activity of the OOM-related 1 protein was analyzed using methods and techniques known in the art. Specifically, sodium-dependent phosphate uptake was measured in opossum kidney (OK) cells according to the methods described in Cai et al. (1994) *New Engl. J. Med.* 330:1645-1649. Briefly, OK cells were cultured until becoming confluent, harvested and then re-seeded at a density of  $1 \times 10^5$  cells per 24 well dish. The cells were then re-grown for several days past the time they become confluent and then re-fed with medium containing the OOM-related 1 protein as well as control conditioned medium from cells that had been transfected with an the expression vector with no cloned insert. In addition, the kidney cells were treated with conditioned medium from mammalian cells transfected with the serine protease 11 gene and with both the serine protease 11 conditioned medium and also the OOM-related 1 protein conditioned medium. After the incubation period extending from 3 to 48 hours, the medium was removed and the plated cells were re-fed with transport medium containing  $^{32}\text{P}$ -labeled dibasic potassium phosphate and incubated at 37°C for 5 minutes. The cells were then washed, harvested and radioactivity measured via a scintillation counter to monitor uptake of  $^{32}\text{P}$ .

Results of the OK phosphate transport assay performed with conditioned medium containing the OOM-related 1 protein and control medium without

recombinant protein showed that conditioned medium that contained the OOM-related 1 protein induced a small reduction in phosphate uptake by the OK cells in comparison with control samples that did not contain this factor. Treatment of the OK cells with both OOM-related 1 protein conditioned medium and also serine protease 11 5 conditioned medium induced an even greater decrease in phosphate uptake by the OK cells. In contrast, treatment of OK cells with only serine protease 11 conditioned medium did not reduce phosphate uptake by OK cells.

These results indicate the OOM-related 1 protein is active in modulating phosphate uptake by kidney cells in combination with the serine protease 11 protein. 10 Thus the OOM-related 1 protein and the gene that encodes this protein are useful targets for modulating phosphate metabolism as well as the symptoms associated with diseases characterized by aberrant phosphate transport such as oncogenic osteomalacia.

It is to be understood that while the invention has been described in conjunction 15 with the above embodiments, that the foregoing description and the following examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

CLAIMS

What is claimed is:

5        1. An isolated polynucleotide encoding oncogenic osteomalacia-related polypeptide (OOM1) or an analog or variant thereof, wherein the polynucleotide comprises or corresponds to a polynucleotide selected from the group consisting of:

10            a) polynucleotide comprising or corresponding to SEQ ID NO:1 or its complement;

                  b) a polynucleotide that hybridizes under stringent conditions to the polynucleotide of SEQ ID NO:1 or its complement;

                  c) a polynucleotide having greater than 80% sequence homology to SEQ ID NO:1 or its complement;

                  d) a polynucleotide having greater than 90% sequence homology to SEQ ID NO:1 or its complement; and

15            e) a polynucleotide having greater than 95% sequence homology to SEQ ID NO:1; or its complement.

                  f) a polynucleotide comprising nucleotides 52 -1629 of SEQ ID NO:1 or its complement;

20            g) a polynucleotide that hybridizes to nucleotides 52-1629 of SEQ ID NO:1 or its complement;

                  h) a polynucleotide having greater than 80% sequence homology to nucleotides 52-1629 of SEQ ID NO:1 or its complement;

                  i) a polynucleotide having greater than 90% sequence homology to nucleotides 52-1629 of SEQ ID NO:1 or its complement;

25            j) a polynucleotide having greater than 95% sequence homology to nucleotides 52-1629 of SEQ ID NO:1 or its complement;

                  k) a polynucleotide comprising nucleotides 100-1629 of SEQ ID NO:1 or its complement;

30            l) a polynucleotide that hybridizes to nucleotides 100-1629 of SEQ ID NO:1 or its complement;

                  m) a polynucleotide having greater than 80% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement;

- n) a polynucleotide having greater than 90% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement; and
- o) a polynucleotide having greater than 95% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement.

5

2. An isolated polynucleotide encoding a oncogenic osteomalacia-related polypeptide, selected from the group consisting of:
  - a) the amino acid sequence shown in SEQ ID NO:2;
  - b) the amino acid sequence shown in SEQ ID NO:2 from amino acid 17 to 10 525; and
  - c) an analog of a) or b) having conservative amino acid substitutions.

3. An isolated polynucleotide encoding a mutein of OOM1 wherein the mutein has the amino acid sequence of SEQ ID NO:2 and non-conservative amino acid 15 substitutions.

4. An isolated fragment of the polynucleotide of claims 1, 2 or 3, wherein the fragment comprises a sequence selected from the group consisting of nucleotides: 1-152; 1-252; 1-352; 1-452; 1-552; 1-652; 1-752; 1-852; 1-952; 1-1052; 1-1152; 1-1252; 1-1352; 52-152; 52-252; 52-352; 52-452; 52-552; 52-652; 52-752; 52-852; 20 52-952; 52-1052; 52-1152; 52-12552; 52-1352; 100-152; 100-252; 100-352; 100-452; 100-552; 100-652; 100-752; 100-852; 100-952; 100-1052; 100-1152; 100-12552; 100-1352; 52-788; and 789-1629, or its respective complement.

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5. A polypeptide encoded by the polynucleotide of any of claims 1-4.

6. An isolated genomic DNA that transcribes the polypeptide of claim 5.

7. A gene delivery vehicle comprising a polynucleotide of any of claims 1-4, or

30

- 6.

8. A host cell comprising a polynucleotide of any of claims 1-4 or 6.

- n) a polynucleotide having greater than 90% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement; and
- o) a polynucleotide having greater than 95% sequence homology to nucleotides 100-1629 of SEQ ID NO:1 or its complement.

5

- 2. An isolated polynucleotide encoding a oncogenic osteomalacia-related polypeptide, selected from the group consisting of:
  - a) the amino acid sequence shown in SEQ ID NO:2;
  - b) the amino acid sequence shown in SEQ ID NO:2 from amino acid 17 to 10 525; and
  - c) an analog of a) or b) having conservative amino acid substitutions.
- 3. An isolated polynucleotide encoding a mutein of OOM1 wherein the mutein has the amino acid sequence of SEQ ID NO:2 and non-conservative amino acid 15 substitutions.
- 4. An isolated fragment of the polynucleotide of claims 1, 2 or 3, wherein the fragment comprises a sequence selected from the group consisting of nucleotides: 1-152; 1-252; 1-352; 1-452; 1-552; 1-652; 1-752; 1-852; 1-952; 1-1052; 1-1152; 1-1252; 1-1352; 52-152; 52-252; 52-352; 52-452; 52-552; 52-652; 52-752; 52-852; 20 52-952; 52-1052; 52-1152; 52-12552; 52-1352; 100-152; 100-252; 100-352; 100-452; 100-552; 100-652; 100-752; 100-852; 100-952; 100-1052; 100-1152; 100-12552; 100-1352; 52-788; and 789-1629, or its respective complement.
- 25 5. A polypeptide encoded by the polynucleotide of any of claims 1-4.
- 6. An isolated genomic DNA that transcribes the polypeptide of claim 5.
- 7. A gene delivery vehicle comprising a polynucleotide of any of claims 1-4, or 30 6.
- 8. A host cell comprising a polynucleotide of any of claims 1-4 or 6.

20. A solid phase support comprising a polynucleotide selected from any of claims 1-4, or 6.

5                   21. An array of probes comprising a polynucleotide of any of claims 1-4, or, attached to a chip.

22. The array of claim 21, which comprises at least 100 polynucleotides.

10                 23. A method for detecting a cell expressing oncogenic osteomalacia-related polypeptide OOM1, comprising contacting a suitable sample with a probe of claim 13 under conditions of moderate hybridization stringency and detecting any hybridized, complementary polynucleotides, thereby detecting the cell.

15                 24. A method for detecting a cell expressing oncogenic osteomalacia-related polypeptide OOM1, comprising contacting a suitable sample with a primer of claim 13 under conditions of moderate hybridization stringency and amplification of complementary polynucleotides, and detecting any amplified polynucleotides, thereby detecting the cell.

20                 25. A method for detecting a neoplastic cell associated with oncogenic osteomalacia, comprising contacting a suitable sample derived from a cell with a probe of claim 13 under conditions of moderate hybridization stringency and detecting any hybridized, complementary polynucleotides, thereby detecting the oncogenic osteomalacia-associated neoplastic cell.

25                 26. A method for detecting a neoplastic cell associated with oncogenic osteomalacia, comprising contacting a suitable sample derived from a cell with a primer of claim 13 under conditions of moderate hybridization stringency and amplification of complementary polynucleotides, and detecting any amplified polynucleotides, thereby detecting the oncogenic osteomalacia-associated neoplastic cell.

27. A method for modulating the phenotype of a neoplastic cell associated with oncogenic osteomalacia, comprising altering the expression of a polynucleotide of any of claims 1-4, or 6 within the cell.

5 28. The method of claim 27, further comprising delivering to the cell an effective amount of serine protease 11.

29. A method of modulating bone mineralization comprising altering the expression of a polynucleotide of any of claims 1-4 or 6 within a cell.

10 30. The method of claim 29, further comprising delivering to the cell an effective amount of serine protease 11.

15 31. A method of treating a disease characterized by abnormal bone mineralization comprising altering the expression of a polynucleotide of any of claims 1-4 or 6 within a cell.

32. The method of claim 31, further comprising delivering to the cell an effective amount of serine protease 11.

20 33. A method for modulating renal phosphate transport, comprising altering the activity of a polypeptide encoded by a polynucleotide of any of claims 1-4, or 6 within a cell.

25 34. The method of claim 33, further comprising administering to the cell an effective amount of serine protease 11.

30 35. A method of treating an oncogenic osteomalacia-associated symptom, comprising altering the expression of a polynucleotide of any of claims 1-4, or 6 within an OOM-associated neoplastic cell.

36. The method of claim 35, further comprising administering to the cell an effective amount of serine protease 11.

37. The method of 35, wherein the symptom is selected from hypophosphataemia, phosphaturia, low serum concentrations of 1,25-dihydroxyvitamin D, or osteomalacia.

5

38. A method for improving an oncogenic osteomalacia-associated symptom, comprising altering the activity of a polypeptide encoded by a polynucleotide of any of claims 1-4, or 6.

10

39. The method of claim 38, further comprising altering the activity of serine protease 11 within the cell.

15

40. The method of 38, wherein the symptom is selected from hypophosphataemia, phosphaturia, low serum concentrations of 1,25-dihydroxyvitamin D, or osteomalacia.

20

41. A method for treating a phosphate homeostasis-related disease, comprising altering the activity of a polypeptide encoded by a polynucleotide of any of claims 1-4, or 6.

25

42. The method of 41, wherein the disease is selected from X-linked hypophosphataemia rickets, oncogenic osteomalacia, rhabdomyolysis, cardiomyopathy, tumoral calcinosis or renal failure.

30

43. A method for modulating the phenotype of a cell comprising altering the expression of the polynucleotide of any of claims 1-4, or 6, within the cell.

44. A method of screening for candidate therapeutic agents that modulate the expression of a polynucleotide of any of claims 1-4, or 6 comprising contacting a test agent with a target cell expressing the polynucleotide, and monitoring expression of the polynucleotide, wherein the test agent which modifies the expression of the polynucleotide is a candidate therapeutic agent.

45. The method of claim 44, wherein the test cell also expresses serine protease

11.

46. The method of claim 44, wherein the candidate agent is a biological or  
5 chemical compound selected from polypeptide, polynucleotide, ribozyme, or small  
organic molecule.

47. A method of screening for candidate agents capable of altering the biological  
activity of a polypeptide encoded by a polynucleotide of any of claims 1-4, or 6,  
10 comprising contacting a test agent with a target cell expressing of the  
polynucleotide, and monitoring activity of the expressed polypeptide product,  
wherein the test agent which modifies the activity of the polypeptide is a candidate  
agent.

15 48. The method of claim 47, wherein the candidate agent is a biological or  
chemical compound selected from polypeptide, polynucleotide, ribozyme, or small  
organic molecule.

49. The method of claim 47, wherein the test cell also expresses serine protease

20 11.

50. A database of polynucleotides comprising the sequences of at least one  
polynucleotide of any of claims 1-4, or 6 in computer readable form.

25 51. A method of analyzing an expressed gene in a cell comprising comparing a  
transcript isolated from the cell with the database of claim 50.

30 52. A method of analyzing the effect of an agent on the expression of at least one  
gene in a cell, comprising comparing at least one transcript(s) of the gene(s) isolated  
from the cell with the database of claim 50.

53. A computer-readable medium having stored thereon the database of claim  
50.

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